Chapter 3

Bone Biology and Variation

Bone, as a connective tissue, and bones, as elements, may be studied on several hierarchical levels. Information derived from many skeletons may be used in reconstructing **population** biology (Chapter 21). Study of the various elements of a single skeleton may be used to elucidate the biological aspects of an **individual** (Chapters 18 and 19). Such assessments are built on a foundation that emphasizes the identification of the bony **elements** that constitute each human skeleton (Chapters 4–13). Before embarking on a systematic consideration of human skeletal elements, it is useful to consider bone biology. This chapter should therefore be considered an essential stepping stone to the descriptive and interpretive chapters that follow.

It is important to note the multiple functions of bone as a tissue and of bones as organs. Bones act as essential mechanical components of the **musculoskeletal system**. They serve to protect and support soft tissues; to anchor muscles, tendons, and ligaments; and as the rigid levers that muscles operate to produce movement. Bones also function as physiologically critical centers for the production of blood cells, as storage facilities for fat, and as reservoirs of important elements such as calcium (essential for blood clotting and muscle contraction). Bone as a tissue is adapted to these functions. The varied mechanical and physiological functions of bones as organs are intimately related to both the gross and microscopic (including molecular) structure of bone tissue, which we review in this chapter.

Bone is a dynamic tissue that allows for growth during **ontogeny** (development) of the individual. It is shaped and reshaped by cells that reside within it. Because of this, the gross shape, or **morphology**, of bones can be altered during life. The shape and size of bones and teeth can also vary dramatically between individuals. Before introducing bone biology at the level of the molecule, the cell, and the gross element, it is critical that we examine a property of all biological structures, the property of **variation**. Understanding and appreciating variation in bony and dental gross anatomy is critically important in any work with the human skeleton.

3.1 Variation

If we were to take a random sample of 50 male and 50 female living individuals from various human populations, it would be easy to establish physical characteristics that would allow each person to be recognized individually. Variation would be employed in sorting among individuals within the population, and only in the rare instance of identical twins would there be much difficulty in distinguishing different people. This is because the human species, like other species, exhibits variation. This variation extends to the teeth and bones of the skeleton.

In identifying our friends and acquaintances, we make use of our ability to recognize variation. Without variation in physical features it would be impossible for us to identify one another at the group and individual levels. In fact, our use of soft tissue variation seems so natural that we take it for granted. Oddly enough, however, the amount of variation in the hard tissues of the body is often not anticipated by students of osteology. Shape and size in human bones and teeth vary widely, and analysis of this variation makes human osteology simultaneously challenging for the beginner and useful for the professional.

There are four major factors leading to variation in human skeletal anatomy. One source of this variation is **ontogeny**, or growth. A great deal of skeletal variation in size and shape is observed along the continuum of growth between fetus and adult. This variation can be used by the osteologist in determining the age at death from skeletal remains. In Chapter 18 we discuss how such analyses are conducted.

A second source of skeletal variation in humans is the sex of the individual. Humans are moderately **sexually dimorphic** in body size, and in any given skeletal population this dimorphism is manifested in the relatively smaller size of female bones and teeth. This size variation is accompanied by shape variation, which allows certain skeletal elements to be used in determining the sexual identity of prehistoric remains. In Chapter 18 we discuss sex determination of osteological material.

A third type of variation is **geographic**, or **population-based**. Different human groups vary in many skeletal and dental characteristics. This geographic variation can be employed to assess the geographic (sometimes called racial) affinity of skeletal remains. In Chapters 18 and 21 we consider the use of this kind of variation in the study of past and present human populations.

Finally, even individuals of the same age, sex, and population differ in anatomy; apart from most identical twins, no two people are identical in their external size and shape. Skeletal elements are not exceptions to this rule. Normal variation between different individuals of the same age, sex, and population is called **individual**, or **idiosyncratic, variation**. This variation can be substantial, but it is too often overlooked. Figure 3.1 illustrates the influences of ontogeny, sex, and idiosyncrasy on variation in the talus, one of the bones of the ankle.

A profusion of classifications of fossils has been created by the failure to appreciate normal skeletal variation in modern species and the failure to understand the principles and goals of taxonomy (White, 2009b). In the resulting forest of family trees, ill-conceived species and genera are hung like ornaments. Normal variation within closely related **extant** (modern) species must guide our expectations of variation in species whose members lived in the past. For this reason, osteologists unfamiliar with normal variation in the present are inclined to misinterpret similar variation in the past as indicating multiple species. To help avoid such misinterpretation, the paleontologist must become familiar with variation in modern humans and their closest relatives, the great apes, by studying large skeletal collections. In assessing any skeletal element, note ontogenetic changes in shape and size by studying different individuals who died at different ages. To assess normal variation in the adult skeleton arising from the other sources identified previously, try to examine a large, mixed-sex sample of individuals. Figures 3.1, 3.2, 3.3, and 3.4 illustrate variation in size and shape in single-site, balanced-sex samples from prehistoric California.

The reality of dimensional (size) and morphological (shape) variation in the hard tissues of individuals makes **typology**, the practice of choosing a single individual to characterize a species, a particularly unsuitable approach to the study and understanding of human osteology and evolution. Yet, to illustrate basic points of anatomy and identification it is necessary to begin somewhere. In using this book's chapters on identification (Chapters 4–13), the student should think of the skeletal elements chosen for illustration as representative, but never as typical. There is no "typical" individual. We illustrate variation in skeletal size and shape in this book to reinforce the fact that such variation in biological structures is normal and to be expected.

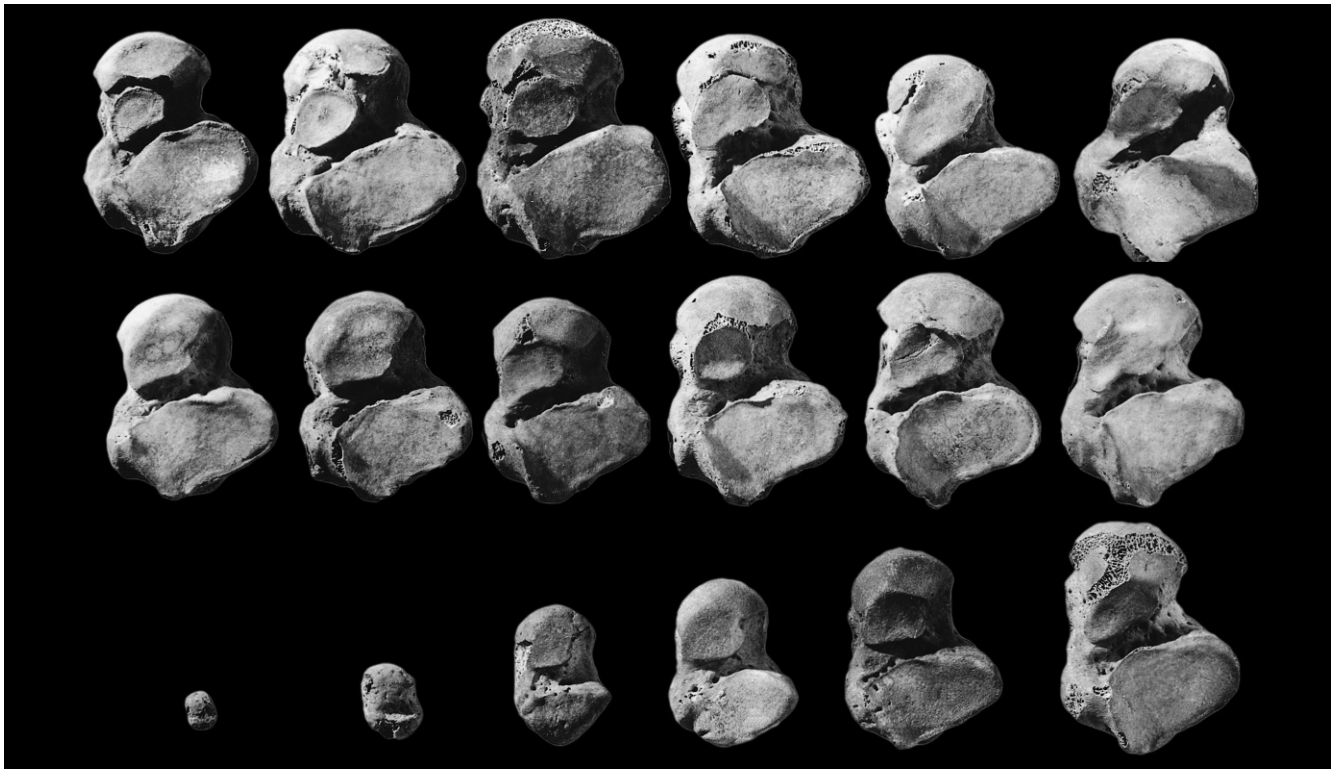

Figure 3.1 **Types of variation.** Normal variation in a bone of the ankle, the talus (viewed from the inferior, or lower, aspect; the anterior, or front, surface of each bone is toward the top of the page). All of the tali shown here were selected from a single-site skeletal sample of 50 prehistoric Californians to illustrate skeletal variation attributable to age, sex, and idiosyncrasy. All specimens are from the left side except for the specimen in the upper right corner, which has been mirrored for easier comparison. One-half natural size.

Top: **Idiosyncratic variation.** Six adult male tali chosen from a sex-balanced sample of 50 adult individuals. Variation in this series is seen in the size and shape of the overall bone outline as well as in the proportions of the parts of the bone and in the topography of the various surfaces. Such variation is common in human skeletal remains.

Middle: **Sex variation.** Three adult female *(left)* and three adult male *(right)* specimens chosen at random from a sex-balanced sample of 50 adult skeletons. *Homo sapiens* is a primate species whose sexual dimorphism in body size is moderate by primate standards, less than the gorilla, but more than the common chimpanzee. Chapter 19 considers sexual dimorphism in the human skeleton.

Bottom: **Ontogenetic variation.** The specimen at the far left is a talus from a newborn child. Tali from individuals at ages 1.5, 6, 10, 12, and 18 years show ontogenetic changes in size and shape of this skeletal element.

3.2 A Few Facts about Bone

Bone — one of the strongest biological materials in existence, particularly in terms of bearing weight (its compressive strength) — is the main supporting tissue of the body. During human running, the bones of the knee joint are loaded with a force in excess of five times the weight of the entire body. Yet, despite its great strength, bone is a very lightweight material. The skeleton itself constitutes less than 20% of the weight of the entire body, whereas a framework of steel bars performing the same mechanical functions as the human skeleton would weigh four to five times more. Bone is a **composite** material, formed of protein (collagen) and mineral (hydroxyapatite). Bone differs from steel because it is a living tissue that can repair and reshape itself in response to external stresses. More detailed reviews of the physical, geometric, and mechanical properties of bone as a tissue, and bones as organs, are provided by Burr (1980) and Currey (1983, 2002).

Bone is the virtually universal rigid underpinning of the musculoskeletal system, and must therefore routinely resist compression, tension, shear, bending, and torsion during the lifetime of

Figure 3.2 **Normal variation.** Adult human clavicles (collarbones) selected to illustrate the total range of variation in size, outline, and topography in a single-site, sex-balanced sample of 80 normal prehistoric Californians. Note the variation in overall size, in the shape of the bone, and in the topography of the surface. This kind and amount of variation should be expected in any normal sample of similar age and mixed sex composition. These right-side specimens are shown in inferior view, with the lateral (arm end) toward the top of the page. One-half natural size.

an individual. In 1869 the German surgeon Julius Wolff formulated a physiological "law" that today bears his name. Wolff observed that bones are living, highly vascularized, structures that can change shape during life (**remodel**), and hypothesized that such changes would in some way systematically improve their capacity to resist such external loading. Simply put, Wolff's Law (referred to as the "law of bone transformation" in 1883) holds that bone is deposited where it is needed and resorbed where it is not needed.

This view that skeletal responses deploy bone in mechanically beneficial ways became generally assumed to underlie not only minor, local changes in the skeleton, but extensive developmental changes that contributed to overall bone form. While extensive experimental research has demonstrated that bone is certainly responsive to loading, such responses have been found to be highly complex and difficult to understand. In addition, more recent investigation of the underlying factors responsible for bone form has demonstrated an overwhelming influence of genetic factors (especially transcription factors specific to growth regions within the bone) in determining overall form. Therefore, natural selection is still the primary key to exploring how and why bones assume their adult shape. Before considering how bones operate at the molecular, cellular, and gross anatomical levels, it is important that we understand the roles of bones in the musculoskeletal system.

3.3 Bones as Elements of the Musculoskeletal System

In the most basic terms, the musculoskeletal system is a system of bony levers operated by **muscles**. Any connection between different skeletal elements is called a **joint**. Bones in the skeleton **articulate** at joints and are connected to one another by means of **ligaments** and **cartilage**. Cartilage is a tough and dense but elastic and compressible connective tissue. Bones are moved by muscles acting directly on the bones or indirectly via **tendons**, which are closely packed par-

Figure 3.3 **Normal variation.** Adult human femora (upper leg bones) selected to illustrate the size and shape variation encountered in a single-site, sex-balanced sample of 100 normal prehistoric Californians. There is considerable variation between specimens shown here in size, robusticity, markings of muscle attachments, and proportions and angles of the different parts of each femur. These left-side specimens are shown in posterior view, with the superior (top) end of the bone toward the top of the page. One-third natural size.

allel bundles of collagen fibers. Movement at the joints is controlled and limited by the shapes of the articular surfaces and by ligaments that bind the joints together and prevent dislocation (Figure 3.5).

The hip, elbow, knee, and thumb joints are all examples of freely moving joints called **synovial joints**. The surfaces of the bones participating in synovial joints are coated with a thin (usually 1–5 mm) layer of slick, articular cartilage called **hyaline cartilage**. The area between the adjacent bones is the **joint cavity**, a space lined by a membrane that secretes a lubricant called **synovial fluid**, which resembles egg white in consistency. This fluid nourishes cartilage cells of the joint and is confined to the joint by the fibrous **joint capsule**, a sac made of connective tissue. It is reinforced by ligaments connecting to the periosteum of the articulating bones (Section 3.4). The combination of hyaline cartilage coating the bone surfaces and synovial fluid lubricating these surfaces gives synovial joints durability with smooth movement and low friction.

Synovial joints are often classified according to the geometric properties of the articulation. The hip joint is a **spheroidal**, or **ball-and-socket** joint, with the hemispherical femur head fitting

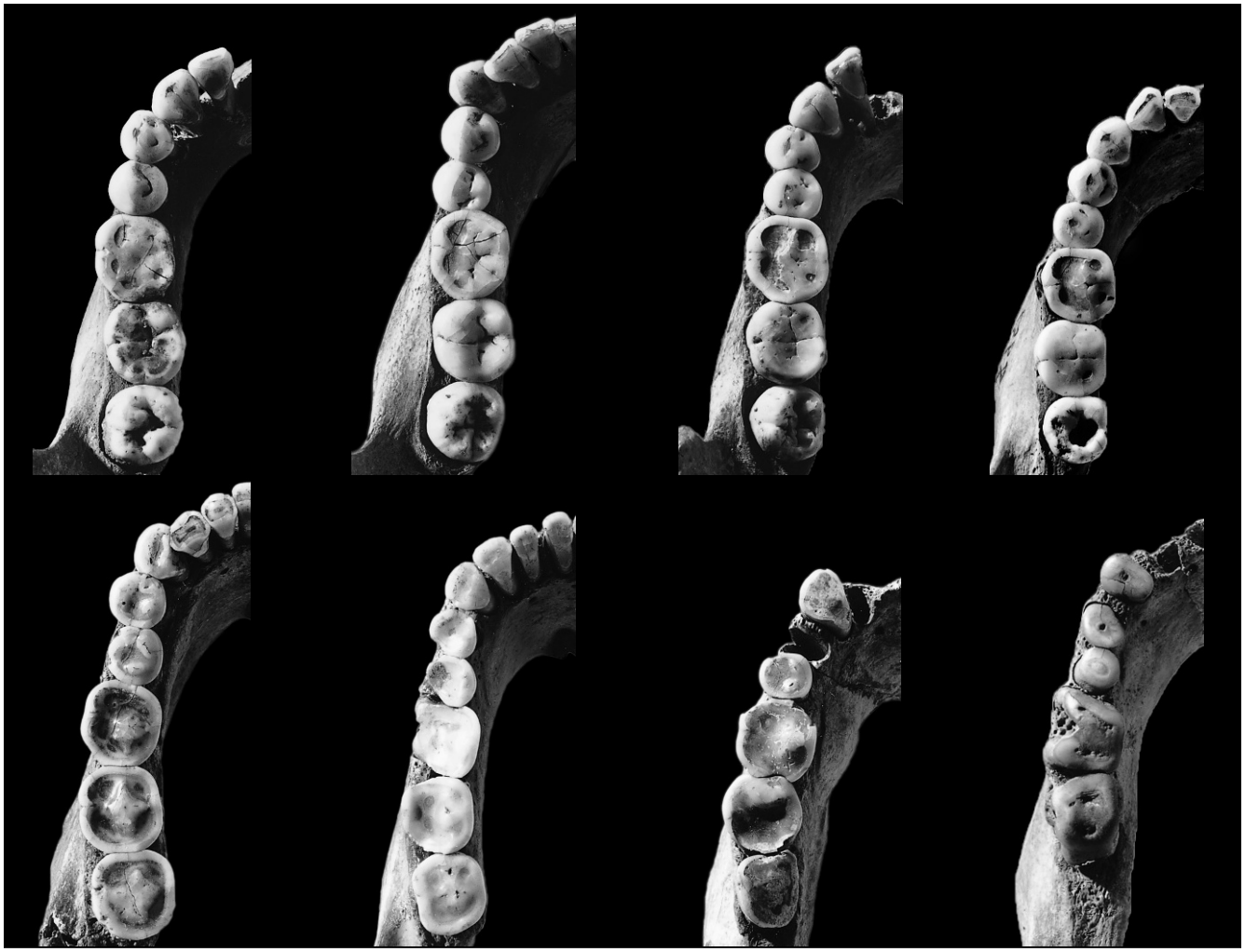

Figure 3.4 **Dental variation due to age (reflected in wear), sex, and idiosyncrasy.** The tooth rows shown here were selected from a single-site, sex-balanced skeletal sample of 60 prehistoric (Late Horizon) Californians. Note the differences in wear, size, and shape of the teeth as well as the variation in tooth row curvature and length.

into the acetabulum, a cavity in the pelvis. This joint structure allows movement in many directions. The elbow and knee joints are called **hinge** joints because they allow a hinge-like movement limited mostly to one plane. The joint at the base of the thumb is called a **saddle-shaped**, or **sellar**, joint because of its shape. It allows movement in two basic directions. **Planar** joints allow two bones to slide across one another, examples of which can be found in the wrist and the arch of the foot.

Because of their mobility, synovial joints are the most obvious joints in the musculoskeletal system, but there are two other important joint types in the body: cartilaginous joints and fibrous joints. In **cartilaginous joints** (or **synchondroses**), the articulating bones are united by means of cartilage, and very little movement is allowed. The temporary joints between growth centers (described later) in a single growing bone are cartilaginous. Some of these joints persist in adulthood, such as the cartilaginous connections between the ribs and the breastbone (sternum). A **symphysis** is a type of cartilaginous joint in which the fibrocartilage between the bone surfaces is covered by a thin layer of hyaline cartilage. **Syndesmoses** are tight, inflexible fibrous joints between bones that are united by bands of dense, fibrous tissue in the form of membranes

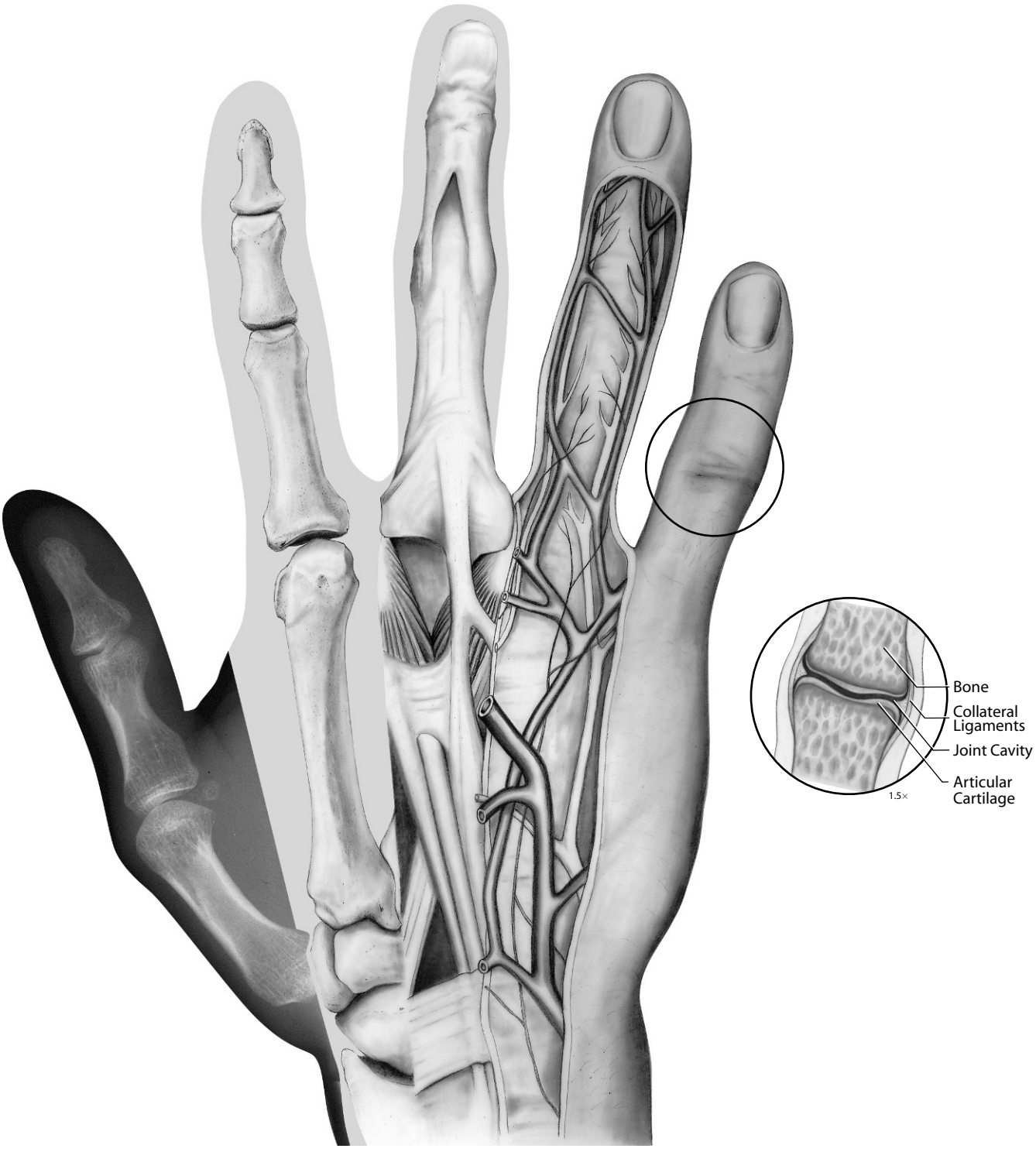

Figure 3.5 **Dorsal view of a human right wrist and hand.** This composite illustration combines the techniques of sectioning (little finger), dissection (ring and middle fingers), and radiography (thumb). The bones are embedded in a matrix of soft tissues including skin, nerves, arteries, veins, muscles, ligaments, tendons, and joint cartilage. In the assessment of external bone morphology, neither the soft tissue matrix nor the internal structure should be forgotten.

or ligaments; an example is the joint at which the two lower leg bones (tibia and fibula) articulate above the ankle (the distal tibiofibular articulation). **Cranial sutures** are fibrous joints of the skull; these are interlocking, usually tortuous joints in which the bones are close together and the fibrous tissue between them is thin. A **gomphosis** is the joint between the roots of the teeth and the bone of the jaws. When any two bony elements fuse together, the result is called a **synostosis**.

Movement of the skeleton takes place, for the most part, at synovial joints. This movement is caused by the muscles, which work by contracting across joints between bones. Muscles usually attach to two different bones, but they may attach to several. Most muscles are connected to bones via **tendons**. **Ligaments** are cords, bands, or sheets of collagenous bundles that extend between and bind the bones forming a joint. Ligaments resist tension, thereby strengthening the joint and permitting only movements compatible with the function of the joint.

Muscle attachment sites are conventionally identified in relative terms. The site that stays relatively stable during contraction of the muscle is called the **origin**. For the appendages, this is usually the attachment site closest to the trunk. The site that is moved by the contraction of a muscle is termed the **insertion**. For example, the muscles that flex the fingers originate in the anterior compartment of the forearm and insert on the fingerprint side of the finger bones (phalanges). Actions caused by muscles are usually reciprocal. At the elbow joint, different muscles cause opposite motions such as **extension** (straightening the arm) and **flexion** (bending the arm). Such muscles are called **antagonists**. Muscles are often identified by the primary action that their contraction causes. Chapters 4–14 introduce some of the major muscles that move the human skeleton and leave traces of their origins and insertions on the bones. For now, we can easily illustrate several basics of the musculoskeletal system with the human hand and arm. Muscles in the forearm are easily palpated as the hand is clenched and opened. These muscles act via tendons, which become very visible across the front and back of the wrist when it is flexed and extended. For example, the *extensor digitorum muscle,* a resident of the forearm, functions in extending the four fingers as it operates via four tendons that cross the wrist.

3.4 Gross Anatomy of Bones

The wide range of bone shapes in the human skeleton seem to defy classification at first. However, the bones in the body are easily partitioned into a few basic, but overlapping shapes. The limb bones and many of the hand and foot bones, usually called long bones, are tubular in shape, with expanded ends (Figure 3.6). The bones of the cranial vault, shoulder, pelvis, and rib cage tend to be flat and tabular. The bones of the ankle, wrist, and spine are blocky and irregular. Despite this variety of external form, the makeup of bones at both the gross and microscopic levels is remarkably constant.

At the gross level, all of the bones in the adult skeleton have two basic structural components: **compact** and **spongy** bone. The solid, dense bone that is found in the walls of bone shafts and on external bone surfaces is called compact, or **cortical**, bone. At joints, compact bone covered by cartilage during life is called **subchondral bone**. This is recognized as smoother and shinier than nonarticular compact bone and lacks the haversian systems described later.

The second kind of bone has a more spongy, porous, lightweight, honeycomb structure. This bone is found under protuberances where tendons attach: in the vertebral bodies, in the ends of long bones, in short bones, and sandwiched within flat bones. This **cancellous**, or **trabecular**, bone is named after the thin bony spicules (**trabeculae**) that form it. The molecular and cellular compositions of compact and trabecular bone tissue are identical; it is only the difference in porosity that separates these gross anatomical bone types.

Areas of trabecular bone in the growing skeleton constitute sites of the **red marrow**, a blood-forming, or **hematopoietic**, tissue that produces red and white blood cells and platelets. The **yellow marrow**, mainly a reserve of fat cells found in the **medullary cavity** (hollow inside the

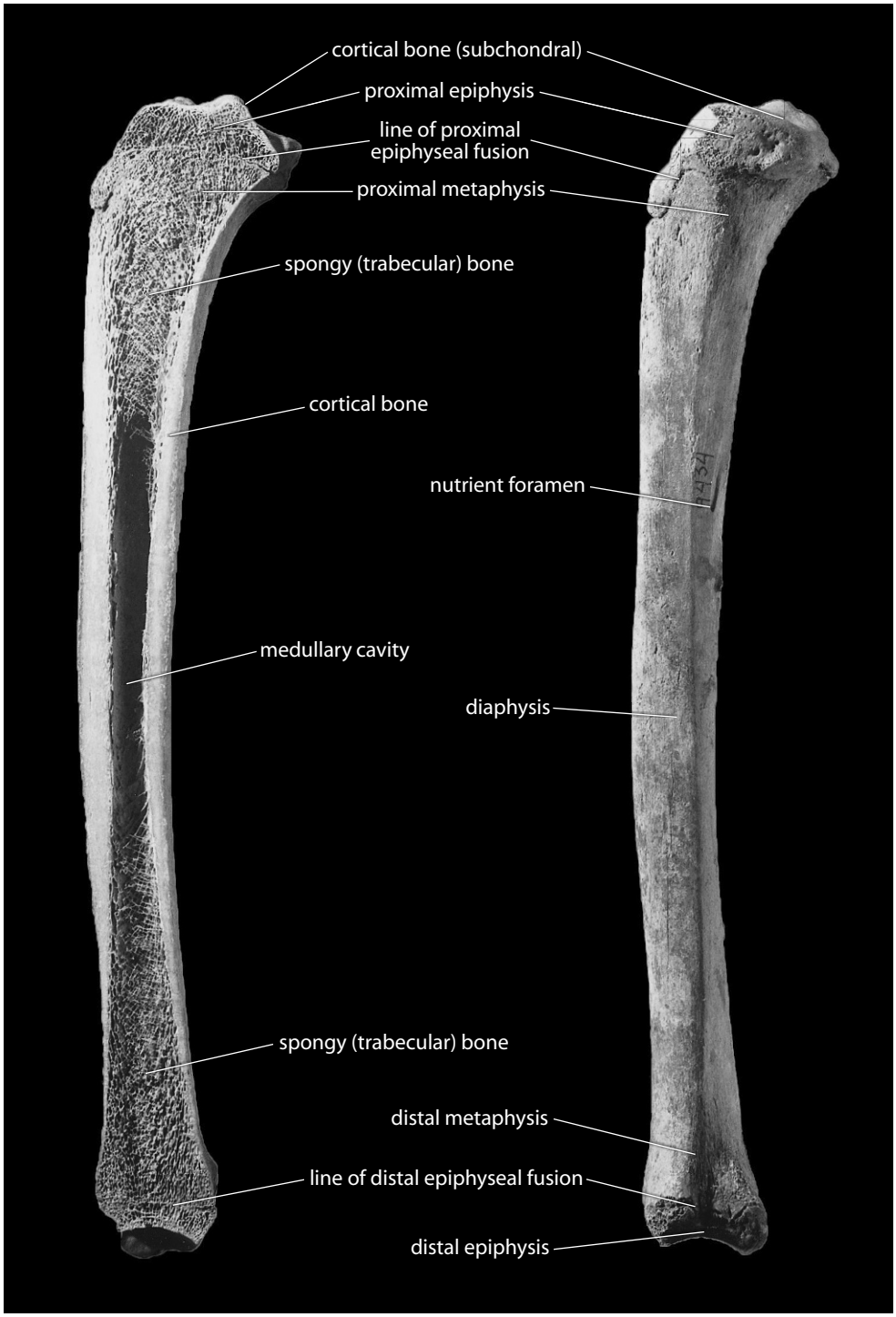

Figure 3.6 **Anatomy of a bone.** A left tibia (shin bone) cut in a parasagittal section to show key elements of the gross anatomy of a typical human long bone. Note the disposition of the compact and spongy bone. One-half natural size.

shaft) of tubular bones, is surrounded by compact bone. During growth, the red marrow is progressively replaced by yellow marrow in most of the long bones. As noted previously, in addition to their role in blood cell production and fat storage, bones function as organs in yet another way: bone tissue represents a calcium reservoir for the body.

Parts of tubular, or long, bones are often described according to the centers of ossification (Section 3.7) that appear during the growth process. The ends of long bones are called the **epiphyses** because they develop from secondary ossification centers of the bone (the articular surfaces of the epiphyses are parts of joints). The shaft of a long bone is called its **diaphysis** because it is the result of the primary ossification center of the bone. The expanded, flared ends of the shaft are called **metaphyses**. A good example of these parts is the knee, where the epiphysis at the knee end of the femur fuses to the metaphysis of the shaft when growth is complete. Some bones have additional growth centers called **apophyses** (or sometimes **traction epiphyses**) that form at the site of tendinous insertions. The iliac crest and the femoral trochanters are good examples of these.

During life, the outer surface of bones is usually covered with a thin tissue called the **periosteum**. This tissue is missing in dry bones, but in life it coats all bone surfaces not covered by cartilage. The periosteum is a tough, vascularized membrane that nourishes bone. Some of the thin fibers of the periosteum penetrate the surface of bone, whereas others intertwine with tendons to anchor muscles to the bone. The inner surface of bones is lined with an ill-defined and largely cellular membrane called the **endosteum**. Both periosteum and endosteum are **osteogenic** tissues — they contain bone-forming cells that are numerous and active during youth. These cells are reduced in number, but remain potentially active, in adulthood. They may be stimulated to deposit bone when the periosteum is traumatized.

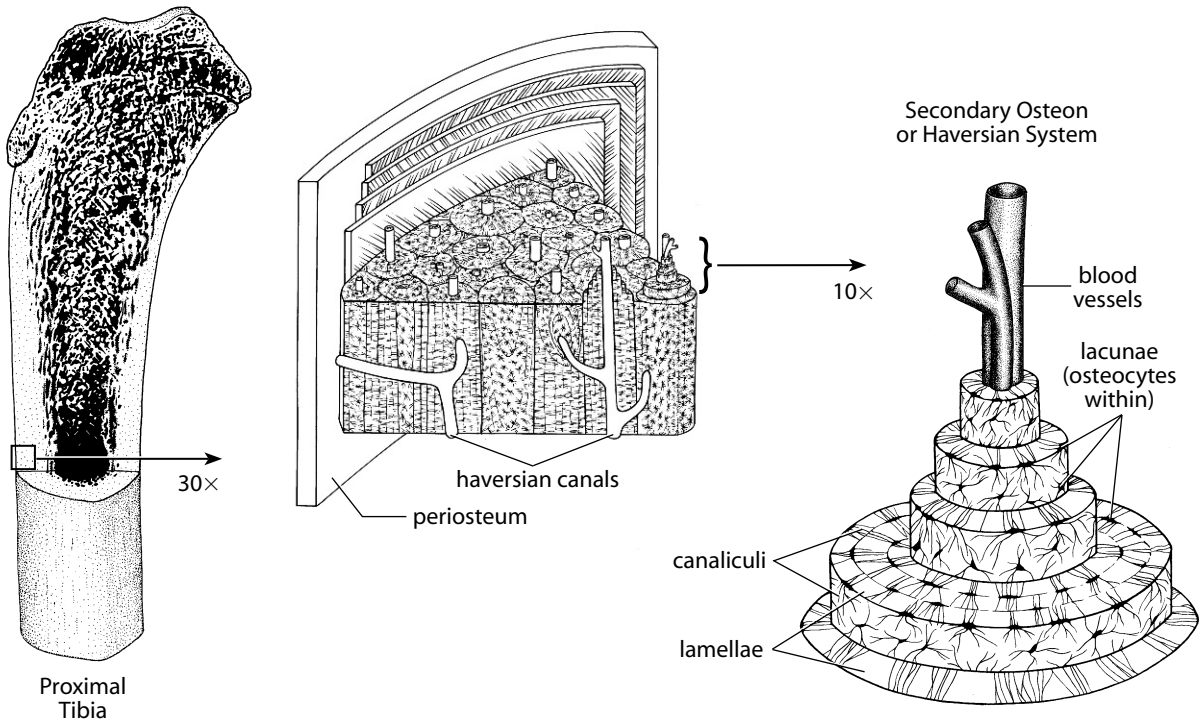

Figure 3.7 **Gross and microscopic structure of bone.**

3.5 Molecular Structure of Bone

We now turn to an assessment of bones at more basic molecular and cellular levels. No matter what shape a bone takes at the molecular level, its tissue is basically the same in all mammals. Bone tissue, like fiberglass, is a composite of two kinds of materials. The first component is a large protein molecule known as **collagen**, which constitutes about 90% of the organic content of bone. Collagen is the most common protein in the body. Collagen molecules intertwine to form flexible, slightly elastic fibers in bone. The collagen of mature bones is stiffened by a dense inorganic filling of the second component, **hydroxyapatite**. In bone, crystals of this mineral, a form of calcium phosphate, impregnate the collagen matrix. This weave of protein and minerals gives bone its amazing properties. The combination of materials is illustrated by two simple experiments. The mineral component gives bone its hardness and rigidity. When soaked in acid to dissolve these minerals, a bone becomes a rubber-like, flexible structure. However, when a bone is heated to combust the organic collagen, or leached out in some archaeological contexts, it becomes extremely brittle and crumbles.

Characterizations of bone at the molecular level give some clues about its physical properties, but it is important to consider that bone as a tissue must be made and maintained by cells. Bone must be responsive to stress, and it must be capable of growth. A look at the structure of bone above the level of the collagen fibril and associated mineral provides insight into these dimensions of bone function.

3.6 Histology and Metabolism of Bone

Histology is the study of tissues, usually at the microscopic level. There are two histological types of mammalian bone: **immature** and **mature**. Immature bone (**coarsely bundled bone** and **woven bone**) is the first kind of bone to develop in prenatal life. Its existence is usually temporary, as it is replaced with mature bone as growth continues. Immature bone is usually formed rapidly and characterizes the embryonic skeleton, sites of fracture repair, and a variety of bone tumors. It has a relatively higher proportion of osteocytes (see later) than mature bone. Woven bone is the more phylogenetically primitive bone type in evolutionary terms. It is coarse and fibrous in microscopic appearance, with bundles of collagen fibers arranged in a nonoriented, random pattern.

Both compact and trabecular portions of adult bones are made of mature, or **lamellar**, bone tissue, named for the orderly, organized structure produced by the repeated addition of uniform lamellae to bone surfaces during appositional growth. Compact bone is composed of dense bone that cannot be nourished by diffusion from surface blood vessels. **Haversian systems**, with their canals and canaliculi (Figure 3.7; described in this section), are the solution to this problem. In contrast, the more porous trabecular bone receives nutrition from blood vessels in surrounding marrow spaces and lacks haversian systems. Normal adult bone, both compact and trabecular, is histologically lamellar bone. Lamellar bone is usually laid down more slowly than woven bone, which it usually replaces.

Microscopic examination of a transverse section of compact bone in, for example, the tibial shaft reveals the internal structure of haversian bone (Figure 3.7). Such a section resembles an end view of a pile of sawed-off tree trunks. The cross section of each "trunk" often shows approximately four to eight concentric rings, known as **haversian lamellae**. A close examination of each lamella would reveal a bed of parallel collagen fibers. Fibers in successive lamellae, however, are oriented in different directions. This alternation of fiber direction adds strength to the structure.

Each "trunk" in the cross section of compact lamellar bone is known as a **haversian system**, or **secondary osteon**. See Figure 3.8a–3.8c for illustrations and descriptions of osteons. These haversian systems measure about 300 μm (0.3 mm) in diameter and are about 3–5 mm in length.

Figure 3.8a **Bone histology.** A comparison of primary and secondary osteons (from Paine and Godfrey, 1997). Polarized lighting, 200×. The primary osteon is an island with lamellar bone streaming around it; the larger secondary osteon intersects the lamellae of primary cortical bone. A primary osteon is composed of a vascular canal without a cement line (because it does not replace pre-existing bone). The cement line (sheath) and lamellar bone organized around the central canal characterize the secondary osteon, which fills a space left by the disappearance of pre-existing bone.

Figure 3.8b **Bone histology.** An intact secondary osteon with several fragmentary osteons. Secondary osteons are products of bone remodeling. Polarized lighting, 200×.

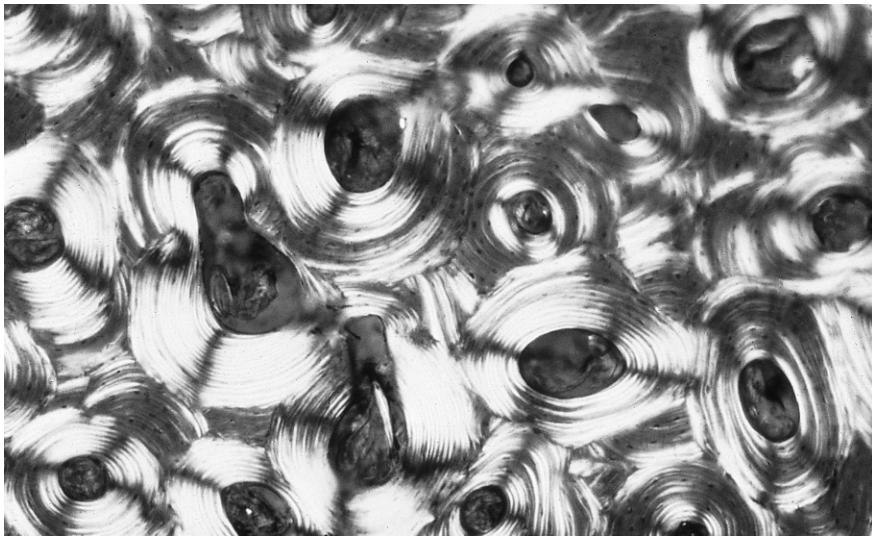

Figure 3.8c **Bone histology.** A crowded field of secondary osteons. The large haversian canals indicate incomplete formation of several osteons. Polarized lighting, 100×. See Chapter 19 for a discussion of how analyses of these microscopic histological structures in bone have been employed in individual age assessment. Courtesy of Robert Paine.

They represent the basic structural unit of compact bone, and their long axes parallel that of the long bone of which they are part. Passing through the core of each haversian system is a hollow **haversian canal**, through which blood, lymph, and nerve fibers pass. Additional smaller canals, the **Volkmann's canals**, pierce the bone tissue obliquely and at right angles from the periosteal and endosteal surfaces to link the haversian canals, creating a network that supplies blood and lymph to the cells of long bones.

Small cavities found within each lamella are called **lacunae**. Each lacuna harbors an **osteocyte**, a living bone cell. Nutrients are transported to these cells through **canaliculi**, minute fluid-filled channels that radiate from the centrally placed haversian canal to lacunae in succeeding lamellae or from one lacuna to others. These channels in bone tissue enable the living cells to survive in a heavily mineralized environment.

Three primary cell types are involved in forming and maintaining bone tissue. **Osteoblasts** are bone-forming cells responsible for synthesizing and depositing bone material. Osteoblasts are often concentrated just beneath the periosteum. They make large quantities of a material known as **osteoid** (pre-bone tissue), an uncalcified organic matrix rich in collagen. Calcification of bone takes place as crystals of hydroxyapatite, the inorganic component of bone, are deposited into the osteoid matrix. Once surrounded by bony matrix, the osteoblasts are called **osteocytes**, cells that reside in lacunae and are responsible for maintaining bone tissue. **Osteoclasts** are responsible for the **resorption** (removal) of bone tissue. All skeletal elements change dramatically during ontogeny and continue to be capable of change in adulthood. Bone formation takes place throughout life. The reshaping, or remodeling, of bone takes place at the cellular level as osteoclasts remove bone tissue and osteoblasts build bone tissue. The opposing processes of bone formation and resorption allow bones to maintain or change their shape and size during growth. Some osteologists distinguish between "modeling" as bone sculpting during growth and "remodeling" as the process of continuous removal and replacement of bone during life.

3.7 Bone Growth

The histological situation described in Section 3.6 accounts for the metabolism of bone and the plasticity of bone in the adult. During ontogeny, however, the skeleton undergoes tremendous growth. Osteocytes do not divide. Because bone matrix calcifies soon after being produced, the tissue cannot undergo further expansion from within. As a consequence, all bone growth is the result of bone deposition on a pre-existing surface. Indeed, bone always develops by replacement of a preexisting connective tissue. Embryologically, bone development (**osteogenesis**, or ossification) occurs in two basic settings. In **intramembranous ossification**, bones, particularly the frontal and parietal bones of the cranial vault, ossify by apposition on tissue within an embryonic connective tissue membrane. Most bones in the skeleton, however, grow through a process known as **endochondral ossification**, in which bones are preceded by cartilage precursors called cartilage models. Early in its development, *in utero*, the skeleton is flexible, but ossification is initiated before birth. Visible elements of the early skeleton are mostly composed of cartilage, a material that is good for rapid growth in an environment where the functions of support are not yet necessary. Cartilage is composed mostly of collagen and, unlike bone, it is flexible and avascular in the adult. The only difference between the two distinct mechanisms of ossification is the environment in which ossification occurs. There is no difference between the kind of bone produced.

Fetal ribs, vertebrae, the basicranium, and limb bones begin as cartilage models. Ossification occurs within the cartilage model as it is penetrated by blood vessels. Growth radiates from the location of the initial penetration, which becomes the **nutrient foramen**. A thin membrane called the **perichondrium** surrounds the cartilage model of the long bone. Osteoblasts just beneath the perichondrium in the fetal long bone begin to deposit bone around the outside of the cartilage shaft. Once this occurs, this membrane is called the **periosteum**, a fibrous connective

tissue, which in turn deposits more bone, layer by layer. As the diameter of the growing long bone shaft increases, osteoclasts on the endosteal surface remove bone and osteoblasts in the periosteum deposit bone. Thus, **appositional growth** allows shaft diameters to enlarge during development. The compact bone of an adult limb bone shaft is periosteal in origin, the original immature shaft having been removed by osteoclasts to form an enlarged medullary cavity. Slow subperiosteal apposition continues throughout life after an adolescent "growth spurt" (Garn, 1972; Garn et al., 1992).

Meanwhile, the developing long bone must also grow in length. During growth, the roughened, porous, usually irregular end of an immature long bone's metaphysis marks the region at which most longitudinal growth occurs. Sandwiched between the metaphysis (the primary center of ossification) and the epiphysis (the secondary center of ossification) during development is a cartilaginous center known as the **growth plate** (**epiphyseal plate**), a tissue layer responsible for bone formation. This plate, a layer of cartilage, "grows" away from the shaft center. The growing cartilage is replaced by bone on the diaphyseal side of the plate. As the individual grows, the epiphyseal plate is pushed farther from the primary growth center of the bone (the shaft), lengthening the bone. Ossification and growth of the bone come to a halt when cells at the growth plate stop dividing, and the epiphysis fuses with the metaphysis of the shaft. Because the ends of the long bone flare, substantial remodeling occurs as the bone lengthens during this process (Figure 3.9).

At 11 weeks before birth there are usually about 800 ossification centers, the "bony pieces" of the skeleton. At birth there are about 350 centers. As a rule, "primary" centers appear before birth, and "secondary" centers appear after birth. The secondary center at the lower end of the femur (upper leg bone) and the one atop the tibia (lower leg bone) begin to appear just prior to

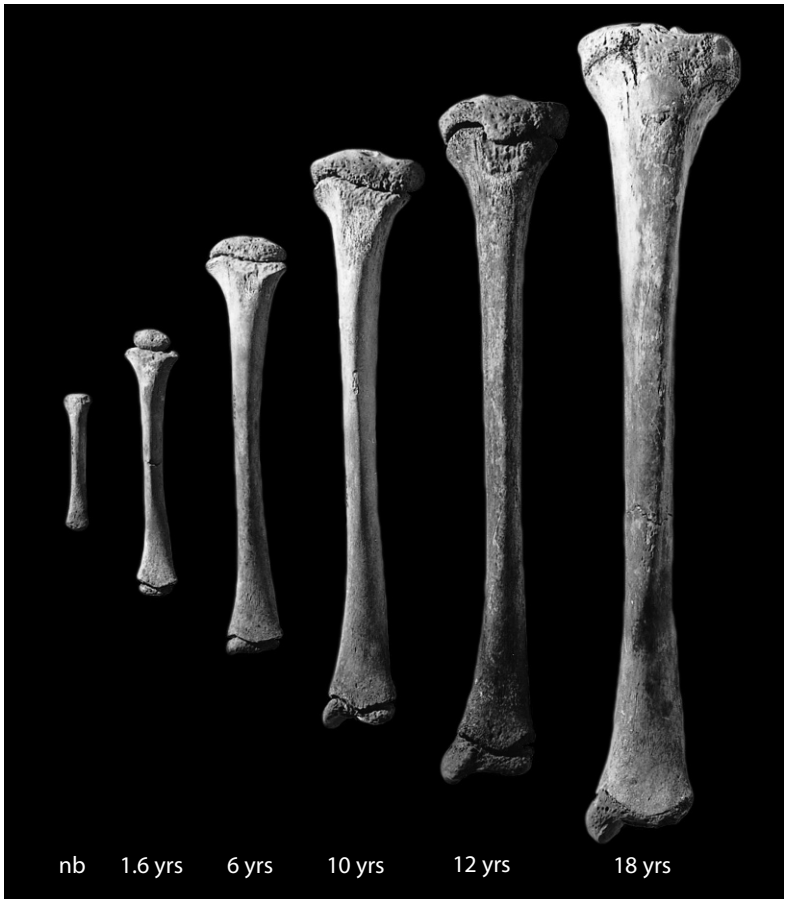

Figure 3.9 **Bone growth.** Growth series for the left human tibia (lower leg bone). The tibia on the far left is that of a newborn child. Larger specimens to the right are from individuals of ages 1.6, 6, 10, 12, and 18 years. Specimens are shown in anterior view, with the proximal (top) end of the bone toward the top of the page. One-fourth natural size.

birth. Most long bones develop two secondary centers in addition to the primary centers of ossification. A few long bones develop a secondary center at one end only, and typical wrist and ankle bones ossify entirely from their primary centers. By adulthood, all of the primary and secondary centers have fused to yield the average adult human complement of 206 elements, the bones of the adult human skeleton. These bones are listed in Chapter 2, Figure 2.1.

3.8 Morphogenesis

Developmental biology is currently a hyperactive field due to the application of molecular techniques to the age-old problem of how form is produced. Discoveries in molecular biology, embryonic limb development, amphibian limb regeneration, cell–cell communication, and the structure and expression of **morphogens**, growth factors, and **homeobox**-containing genes have rapidly advanced knowledge about how form is shaped during ontogeny.

As Müller (1997) points out, "self-construction" and "self-organization" are terms that convey the essential properties of development. All humans start with a single, apparently unstructured cell, the fertilized egg. Embryogenesis follows, and then birth and further development. At the end of the process, the fully developed human is an organism with an intricately wired brain that contains over a hundred trillion synaptic contacts that help make it possible for us to ask how the complex shapes and sizes of the human skeleton are encoded and how that code is translated by cells during development. Cells differentiate, communicate, and interact morphologically and functionally. Together they construct multicellular structures such as bones.

The shaping of form, or **morphogenesis**, from simple, seemingly amorphous, generative, starting cells has puzzled biologists and philosophers alike. A big step toward solving the problem came when it was demonstrated that DNA acted as a genetic code. The genome contains information about how to make distinct proteins, rRNA, tRNA, and how to replicate itself. It contains elements of a spatiotemporal program that controls the order and pattern of gene expression. As Gilbert and Singer (2010) point out, we are still trying to uncover the details of how a developing human is created on the basis of such minimal information (approximately 20,000–25,000 genes in a human). We know that different combinations of genes become effective in time and space in different cells, organs, and body regions. We know that cells interact, influencing each other. For example, a fracture of the adult humerus will stimulate production of bone and healing, but there was no way that the fertilized egg could "know" in advance that any individual's humerus would be fractured at a particular time in adult life.

Basic events in animal development include cell proliferation (recurring cell division), cell differentiation (which occurs in a defined spatial order), and **pattern formation** (the spatiotemporal ordering of molecules, cells, or tissues to form a pattern, which can then develop at different scales). This is the process whereby spatial organization of cell differentiation is controlled. Cells obtain positional information by virtue of their location within a tissue. Cells move, migrate, and die according to genetically determined schedules. All of these events are important in morphogenesis, a process tightly choreographed by highly conserved genes and gene arrays.

Bone develops originally from embryonic mesenchymal stem cells that have a very broad range of development potentialities (they are **pluripotent**), giving rise to fat, muscle, and other cells. Along the road to their differentiation into bone-producing cells, a population of cells with more limited potential is formed. These are only able to proliferate into chondroblasts or osteoblasts. These osteoprogenitor cells persist throughout postnatal life and are found in the endosteum and periosteum. They are most active during bone growth but can be reactivated in adult life when fracture repair is initiated (Section 3.9).

During embryogenesis, an **anlage**, or aggregation of cells indicating the first trace of an organ, forms. Recent work in limb development has begun to unravel the process through which an integrated system of sequentially expressed genes and/or gene arrays guides development of the limb assigning positional address by morphogens (molecules that influence morphogenesis),

growth factors, signaling molecules, and homeoboxes (a family of highly conserved base pair sequences of the DNA that encode small proteins that activate specific genes). The homeobox sequence is preserved with only minor modifications in a wide variety of animals, and is very similar in fruit flies, birds, and mammals.

It is already clear that development of a limb is guided by morphologic data sequestered in highly systematic gene arrays and implemented by stereotyped and largely universal cellular response regimens. For example, implantation of a single acrylic bead soaked in the protein fibroblast growth factor and subsequently placed in the flank of an early chick embryo can trigger the formation of an entire new limb. It now appears that most sculpting of the skeletal frame occurs during the earliest phases of embryogenesis. Once the anlage is formed, further skeletal development appears to be directed primarily by the influence of stress history on gene expression by what may be called "assembly rules." These guide the behavior of each connective tissue cell during this process. For the functional morphologist, these insights into morphogenesis have fundamental implications (Lovejoy et al., 1999, 2003). What this means to the practicing osteologist is that much individual skeletal variation is the product of the interaction of the environment with these assembly rules rather than a direct readout of some gene(s). For example, the expression of an intertrochanteric line on the femur (Section 12.1.1d) represents an individual variation rather than a species-specific, genetically encoded trait.

3.9 Bone Repair

Bones occasionally break, or **fracture**, when subjected to abnormal stresses or when bone is weakened pathologically. The process of repair begins as soon as the fracture occurs. Blood vessels in the haversian canals, the periosteum, and the marrow are usually ruptured by a fracture. Blood flows into the fracture zone and normally forms a **hematoma** (bloody mass) that coagulates as the blood vessels are sealed off. The periosteum is usually torn at the fracture site and pulled away from ends of the broken bones. This stimulates the osteogenic layer of the periosteum to begin forming a **callus**, fracture repair tissue that forms a sort of natural splint. The callus first consists of fibrous connective tissue that bridges the broken bone surfaces, tying them together. Within two days the osteoblasts respond, and the callus is subsequently mineralized to form woven bone, the **primary bony callus.** The primary bony callus takes about six weeks to develop. Later, this woven bone callus is converted to lamellar bone. If orientation of the broken bone ends is close to the original, and if subsequent movement at the fracture site is limited (especially by immobilizing the bone), the callus may become so remodeled that evidence of fracture is eventually present only in radiographs. Further remodeling may completely eliminate any evidence of the fracture. Chapter 19 illustrates some effects of fracture in bone. Recent clinical work has shown that proteins known as bone morphogenetic proteins (BMPs) can be combined with a matrix composed partly of demineralized collagen and applied to serious fractures to speed healing (De Biase and Capanna, 2007).

In a fascinating intersection of applied and basic research, it turns out that these proteins are produced by genes belonging to a very ancient family — genes homologous to those in fruit flies. The nightmarish disease called fibrodysplasia ossificans progressiva (FOP) is a heritable disorder of connective tissue characterized by congenital malformation of the large toes and progressive, disabling endochondral osteogenesis in predictable anatomical patterns. Disease progression brings fusion of adjacent bones of the spine, limbs, thorax, and skull, leading to immobilization (Figure 3.10). Disease flare-ups can occur spontaneously or can be induced by minor trauma such as intramuscular drug injections. This abnormal bone buildup occurs because the FOP patient's white blood cells erroneously manufacture BMP-3, triggering inappropriate heterotopic ("other" + "place") bone growth at sites of injury (Shafritz et al., 1996).

A mutation in the ACVR1 (activin receptor type-1) gene was found to be the cause of FOP (Shore et al., 2006). The ACVR1 mutation (called R206H ACVR1) results in a change to one of

Figure 3.10 **Advanced bony manifestations of fibrodysplasia ossificans progressiva in a 39-year-old man**. See Section 3.9 for details. Courtesy of Fred Kaplan, Mütter Museum, College of Physicians of Philadephia (Shafritz et al., 1996).

the gene's 509 amino acids. In people with FOP, the mutation causes amino acid position 206 to code for the amino acid arginine instead the amino acid histidine. This small change to the ACVR1 gene, an important BMP signaling switch for the cartilage cells in metaphyses, as well as in skeletal muscle, is all that is needed to start turning skeletal muscle into bone and imprison its victims in a "second skeleton." The precise molecular physiology of the R206H ACVR1 mutation is still being investigated, with the hope of finding a treatment and eventual cure for FOP. Such remarkable findings at the intersection of basic research in molecular genetics and applied research in the medical clinic are now commonplace in biology. They hold out the promise to allow a fuller understanding of how skeletal form develops and how broken bones can be healed.

Suggested Further Readings

Active research into bone biology at all levels renders many older texts obsolete. The sources below provide supplementation to the discussion presented above.

Bilezikian, J. P., Raisz, L. G., and Martin, T. J. (2008) *Principles of bone biology* (3rd ed.). San Diego, CA: Elsevier. 1900 pp. in 2 volumes

A comprehensive reference to nearly all aspects of modern bone biology.

Bronner, F., and Farach-Carson, M. C. (2003) *Bone formation*. London, UK: Springer-Verlag. 160 pp.

An edited volume covering the current state of research into the cellular mechanisms of regulation, bone growth, and bone disorders.

Currey, J. (2002) *Bones: Structure and mechanics.* Princeton, NJ: Princeton University Press. 456 pp.

An excellent and approachable text on the biological and mechanical properties of bone.

Fawcett, D. W., and Jensh, R. P. (2002) *Bloom & Fawcett: Concise histology* (2nd ed.). New York, NY: Arnold. 352 pp.

A comprehensive textbook with chapters on cartilage, bone, and teeth, with fine illustrations.

Gilbert, S. F., and Singer, S. R. (2010) *Developmental biology* (9th ed.). Sunderland, MA: Sinauer Associates. 685 pp.

A thorough introduction to the major topics in contemporary developmental biology.

Hall, B. K. (2005) *Bones and cartilage: Developmental and evolutionary skeletal biology.* San Diego, CA: Elsevier Academic Press. 792 pp.

An in-depth review of the major topics in bone and cartilage research. Interesting, accessible, and well-illustrated introduction to both the growth of bone and its evolutionary origins.

Minelli, A. (2009) *Forms of becoming: The evolutionary biology of development.* Princeton, NJ: Princeton University Press. 242 pp.

An accessible introduction to the principles of modern evolutionary developmental biology.

Ogden, J. A. (1990) Histogenesis of the musculoskeletal system. In: D. J. Simmons (Ed.) *Nutrition and bone development.* Pp. 3–36. New York, NY: Oxford University Press.

An excellent review of bone development.

Ortner, D. J. (2003) *Identification of pathological conditions in human skeletal remains* (2nd ed.) San Diego, CA: Academic Press. 645 pp.

This illustrated text includes a good chapter on the biology of skeletal tissues.

Zollikofer, C. P. E., and Ponce de León, M. S. (2010) The evolution of hominin ontogenies. *Seminars in Cell and Developmental Biology* 21: 441–452.

An introduction to the emerging, synthetic field of evolutionary developmental paleoanthropology.